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INFLUENCE OF REACTIVE PHOSPHORUS (RP) CONCENTRATIONS ON OCCURRENCE OF HETEROTROPHIC BACTERIA CAPABLE OF MATTER TRANSFORMATION, INCLUDING PHOSPHORUS IN WATER ENVIRONMENT

WPŁYW STĘŻEŃ REAKTYWNYCH FORM FOSFORU NA WYSTĘPOWANIE W ŚRODOWISKU WODNYM BAKTERII HETEROTROFICZNYCH ZDOLNYCH DO PRZEMIAN MATERII ZAWIERAJĄCEJ FOSFOR

Abstract: This survey has been aimed at estimation of the Vistula water number of heterotrophic bacteria, capable of decomposition of various phosphorus compounds, both organic and inorganic ones, as well as determination of bacteria participation in biogeochemical phosphorus cycle. The studies were conducted from spring 2000 to spring 2001. The water for analyses was sampled from three current sites of the Włocławek Reservoir along former bed of the Vistula River. In this studies has been estimated number of heterotrophic bacteria, capable to release mineral phosphorus from organic and inorganic matter. The result of the conducted study demonstrated that the key role in recovery of biologically available phosphorus amounts is the one of bacteriological phosphatases.

Keywords: phosphorus in water, release of mineral phosphorus from matter, heterotrophic bacteria

Phosphorus is one of the most important elements for both autotrophic and heterotrophic organisms. Phosphoric acid radicals are comprised in ADP and ATP, as well as oxidoreductases NADP, which take part in transfer of energy and phosphoric acid radicals into intermediate products of photosynthesis or respiration. This element is contained in nucleic acids (RNA and DNA).

Environmental shortage of phosphate limits the ecosystem productivity while its excess in water environment leads to eutrophication [1, 2]. Environmental phosphorus can be divided into two major groups of compounds: organic and mineral. Organic phosphorus compounds emerging in water origin mainly from floral and animal deritus, micro-

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biological synthesis products, and possibly sewage [3]. These substances influenced by microorganisms undergo active dephosphorylation with phosphates abstraction. Enzymatic phosphatase play special role in this process [4]. Heterotrophic bacteria and other microorganisms produce alkaline and acidic phosphatases as well as 5'-nucleotidase, *ie* enzymes which release mineral phosphorus from such compounds as nucleotides, phosphosaccharides and phospholipids, contributing to decomposition of organic phosphorus forms [5]. Released phosphate is partially reassimilated by microorganisms, which cause degradation within processes of biochemical decompositions. Plants absorb large part of it. However, in water environments some amount of released phosphate is likely to bind cations of calcium, magnesium, aluminium or iron and produce sparingly soluble phosphates, which sink as deep as to bottom sediments [3]. Microorganisms biochemical activity triggers transformations of inorganic phosphates. This is brought about by acids produced by microorganisms [6]. In case of heterotrophic microorganisms, phosphate transformation is triggered by the impact of produced organic acids or complex-creative factors. Autotrophs are also capable of transforming insoluble phosphates through production of sulphuric and nitric acids.

This survey has been aimed at estimation of the Vistula water number of heterotrophic bacteria, capable of decomposition of various phosphorus compounds, both organic and inorganic ones, as well as determination of bacteria participation in biogeochemical phosphorus cycle.

Material and methods

Samples were taken from spring 2000 to spring 2001. The water for the purpose of physicochemical and microbiological analyses was sampled from three current sites of the Włocławek Reservoir along former bed of the Vistula River.

Site I "Płock" – situated by the road bridge in Płock (632 km of the river reaches). It is typical river site with average flow velocity of ca 0.1 $m \cdot s^{-1}$.

Site II "Dobiegniewo" – in the vicinity of Dobrzyn nad Wisla (660 km of the river reaches). It is a middle section of the reservoir with a vast overflow area. This site has not a typical river nature. The average water flow velocity amounts to ca 0.1 $m \cdot s^{-1}$.

Site III "Włocławek dam" – the samples were taken around 300–400 m before the dam (675 km of the river reaches) from the surface layer as well as the bottom water (site IV) at a depth of ca 11 m, *ie* 0.5 m above the bottom sediment. The average water flow velocity amounts to ca 0.1–0.4 $m \cdot s^{-1}$.

The water for the purpose of analyses was received by means of Patalas' sampler and poured into sterile glass containers. Collected water samples were carried to the laboratory in sealed container at the temperature of ca + 7 °C.

The number of heterotrophic bacteria (CFU) the analysed samples contained was estimated according to grated screening method with application of yeast extract medium. 0.1 cm^3 accordingly dissolved water was delivered to the medium surface and spread with a sterile glass device. The incubation lasted 7 days at 20 °C. The emerged colonies were counted and received numbers were converted into number of heterotrophic bacteria in investigated water considering the material dissolution degree.

Isolation of bacterial strains. Representative strains collection was detached from the cultures that were a base of heterotrophic bacteria number estimation. Each site was represented by ca 20 strains, which were transported into the yeast extract medium bevels. After microbiological material has been generated the bevels were stored at 4 °C yeast for the purpose of further research. Bacterial strains were transferred into fresh yeast extract media every two months.

Concentrations of reactive phosphorus (RP) were estimated according to Hermanowicz [7]

Production of alkaline phosphatase by bacterial strains was analysed by culturing them on the medium containing:

KNO_3 – 0.5 g; $MgSO_4 \cdot 7H_2O$ – 0.4 g; $CaCl_2 \cdot 2H_2O$ – 0.2 g; NaCl – 0.1 g; Fe Cl_3 – 0.01 g; casein hydrolysate – 4.0 g; H_2O (d) – 1000 cm^3; pH – 7.0–7.2.

The medium was distributed among 5 cm^3 test tubes and sterilized at 117 °C for 20 min. Afterwards the medium was populated with investigated strains and incubated at 26 °C for 24 hours. Next step involved addition of 1 cm^3 sterile colourless substrate solution *p*-nitrophenyl phosphate (Sigma) in 1 $mg \cdot cm^{-3}$ concentration, prepared in Tris-HCl buffer (pH 9.0). After further 24 hours of incubation in darkness, the colour of the culture was observed. Yellow colour would have proved a hydrolysis of the substrate into a coloured product as a result of exogenous phosphatases.

Capability of DNA hydrolyse by surveyed strains was tested with application of the medium DNase TEST AGAR (DIFCO). The sterile medium on pans was inoculated with the surveyed strains applying an inoculation loop. The incubation took place at 20 °C for 24–72 hours. 1 M HCl was used for reading off the results. A positive result was deemed emerging of brighter zone around the colony – an evidence for DNA decomposition.

Capability of lecithin decomposition was conducted using test medium [7]

The sterile medium on the Petri dishes was inoculated with the surveyed strains using inoculation loop. The results were read after 2 and 4 days of incubation at 26 °C. A positive result was indicated by cloudiness zones around the colony.

Capability of mineral phosphorus compounds decomposition by bacterial strains was surveyed by culture them on the medium with a content of $Ca_3(PO4)_2$ according to Rodina [8]. The sterile medium on the Petri dishes was inoculated with the surveyed strains using an inoculation loop. The incubation was conducted at 20 °C for 7 days. Starting from 2 days of incubation, the results were read every 2 days. Emerging of brighter zones around the colony indicated a positive result.

Results and discussion

Phosphorus plays a key part in water ecosystems functioning. It is the most important element determining water environment fertility. Therefore the research on the phosphorus biogeochemical cycle are rated among the most essential activities concerning element circulation.

Bacteria constitute a key and dominant factor of mineral compounds and organic matter circulation processes in any environment. Water microorganisms metabolic processes activity and rate determine circulation of series of elements, both in specific ecosystems and on a global scale [9–12]. Heterotrophic bacteria are particularly significant, since they are a vital component of trophy network of natural water environments. These microorganisms are responsible for processes of degradation, mineralization, and disposal of organic matter. Heterotrophic bacteria enable energy flow as well as biogene circulation in water ecosystems [13].

Conducted studies confirmed that the heterotrophic bacteria number in the investigated length of the Lower Vistula within the whole research period ranged from $3.5 \cdot 10^3$ to $145.5 \cdot 10^3$ (Table 1). Such substantial differences of bacteria numbers in the investigated water are results of specific nature of the Vistula. Since the Vistula is the biggest Polish river, it collects waters from a vast area. Furthermore, the water levels in the river as well as the amounts of suspended solids significantly vary depending on the season and precipitation volumes. The organic suspension is a nutritional source of supply for the heterotrophic bacteria, while according to Donderski [14] it is the most vital factor affecting bacteria growth in water bodies. Large numbers of bacteria in surveyed water were observed in the spring 2001. Seasonal increase is connected with water body refill with a fresh nutritional substance carried with waters from spring melt.

Table 1

Numbers of heterotrophic bacteria in investigated samples of water (cells $\cdot$ 10^3 $\cdot$ cm^{-3})

Date of sampling	Site I	Site II	Site III	Site IV
May 2000	21.50	130.00	3.50	45.00
August 2000	49.50	10.50	6.00	7.50
November 2000	78.00	55.00	13.00	42.00
March 2001	108.50	145.50	71.50	32.50
Average	64.38	85.25	23.50	31.75

Apart from estimation of heterotrophic bacteria number, which constituted a background for further investigations, this study included estimation of number of bacteria capable of decomposition of various compounds containing phosphorus.

Many investigations confirmed that heterotrophic bacteria and other microorganisms secrete series of hydrolytic enzymes that able decompose organic matter. At least three primary groups of phosphohydrolytic enzymes commonly produced by water organisms take part in the processes of organic phosphorus derivatives decomposition and disposal. These inculde phosphoesterases, that are alkaline and acidic phosphatases, nucleotidases (mainly 5'-nucleotidase), and nucleases (endo- and exonucleases): RNAse and DNAse that decompose nucleic acids RNA and DNA [4]. Most of them are exoenzymes. Only some of them are likely to occur in the environment in forms of free enzymes or enzymes adsorbed on seston or on mineral particles [15].

A has been demonstrated by former research, on average: 52.42 % (site I); 32.90 % (site II); 45.88 % (site III); 26.66 % (site IV) of bacterial strains produced exogenous phosphatases (Table 2).

Table 2

Occurrence of bacteria capable of various phosphorus forms transformations (in %)

Date of sapling	Production of alkaline phosphatase	DNA hydrolyse	Lecithin decomposition	Mineral phosphorus decomposition
Site I				
May 2000	68.00	28.00	0.00	4.00
August 2000	56.67	26.67	10.00	3.33
November 2000	20.00	44.00	8.00	12.00
March 2001	65.00	55.00	5.00	0.00
Average	52.42	38.42	5.75	4.83
Site II				
May 2000	57.14	46.67	16.67	3.33
August 2000	13.33	9.52	9.52	0.00
November 2000	11.11	44.44	0.00	0.00
March 2001	50.00	25.00	0.00	0.00
Average	32.90	31.41	6.55	0.83
Site III				
May 2000	87.50	75.00	12.50	0.00
August 2000	25.00	0.00	0.00	0.00
November 2000	4.35	30.43	8.70	17.39
March 2001	66.67	20.00	5.00	0.00
Average	45.88	31.36	6.55	4.35
Site IV				
May 2000	22.22	11.11	11.11	0.00
August 2000	25.00	15.00	0.00	0.00
November 2000	29.41	47.06	0.00	0.00
March 2001	30.00	15.00	0.00	0.00
Average	26.66	22.04	2.78	0.00

Production of phosphatases by bacteria is affected by RP concentration in water. RP concentration levels are presented in Table 2. Siuda [15] proved that alkaline phosphatases content varies depending on phosphorus availability in the environment. A rapid increase of alkaline phosphatases activity was observed in the cells of algae and bacteria, which were deprived of phosphorus sources, on more than one occasion. In conditions of excessive phosphorus amounts there were minimum levels of these enzymes. In the presented study we noted, that in March when the concentrations of RP were very low (Table 3), the percentages of bacteria capable of production of alkaline phosphatase were high. In May, the concentrations of RP were also very high, but in late spring, phosphorus is frequently released microbiologically from the sediments into the water depths [2].

Table 3

The concentrations of RP in investigated samples of water [mg · cm^{-3}]

Date of sampling	Site I	Site II	Site III	Site IV
May 2000	0.085	0.810	0.085	0.092
August 2000	0.044	0.112	0.061	0.092
November 2000	0.059	0.056	0.058	0.086
March 2001	0.029	0.036	0.032	0.032

This study included also decomposition of: DNA, lecithin, and mineral phosphorus compounds. According to Rodina [8] many microorganisms that have an enzyme phosphatases conduct hydrolysis of organic compounds with content of phosphorus. It is assumed that there are 15–20 enzymes of that group, which vary with respect to chemical nature of hydrolysed substrates. Phosphatases affect nucleic acid and lecithin beside other enzymes, eventually causing an abstraction of phosphates by series of transition compounds.

The data concerning DNA decomposition analysis confirms that mean contents of those bacteria in waters (sites I-IV) amounted to: 38.42; 31.41; 31.36; and 22.04%, respectively (Table 2).

In the study pertaining to the lake Jeziorak Mały, Donderski et al [16] obtained results, which proved that 27 % of total isolated bacteria were capable of DNA hydrolysis. Present study results concerning contents of bacteria capable of DNA decomposition are very similar.

The data concerning lecithin confirm that this compound was decomposed to a much lower degree by bacteria inhabiting investigated waters. Mean percentages of bacteria capable of lecithin mineralization on sites I–IV waters amounted to 5.75; 6.55; 6.55, and 2.78 %, respectively (Table 2). In the studies, lecithin decomposing bacteria conducted in the same Vistula length Piasecka [17] received following results: November 2000 as much as 77.14 % strains decomposed lecithin; March 2001 – 65.22 %; May 2001 – 66.67 %; July 2001 – 40 %. The differences between results of both studies pertaining to lecithin decomposition abilities can corroborated by justified with various results concerning phosphatases production, which in Piasecka's study amounted to 100 %, whereas in present study were 39.6 %. Results obtained by Wu Gen Fu and Zhoe Xue-Ping [6] proved that only small number of bacterial strains was capable of lecithin decomposition. They made up as little as ca 13 in 1 cm^3.

Analysis of occurrence of bacterial strains capable of mineral phosphorus compounds decomposition suggests that there were few of such strains. In water on individual sites they constituted a minor percentage from 0 to 4.83 % (Table 2). Hence, the role those bacteria play in the Vistula water enrichment with mineral phosphorus forms does not seem significant. Bacterial strains capable of RP release are abundantly represented in bottom sediments – their numbers can reach level of $5 \cdot 10^4$ cells/cm^{-3} [6]. Mentioned authors claim that those strains numbers in water is insignificant – ca. 100 cells/cm^{-3}.

Summing, for capability of transforming various phosphorus forms, both organic and inorganic ones, heterotrophic bacteria are immensely important elements of trophy network within water ecosystems. This study confirms results of Hermandez et al [4] that the key role in recovery of biologically available phosphorus amounts is the one played by bacteriological phosphatases.

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Abstrakt: Celem pracy było oznaczenie liczebności bakterii heterotroficznych w wodzie Wisły, zdolnych do rozkładu różnych związków fosforu zarówno organicznych, jak i nieorganicznych oraz określenie udziału bakterii w cyklu biogeochemicznym fosforu. Badania prowadzono od wiosny 2000 r. do wiosny 2001 r. Próbki wody do badań pobierano z trzech stanowisk nurtowych Zbiornika Włocławskiego wzdłuż dawnego koryta rzeki Wisły. W badaniach oznaczano liczebność bakterii heterotroficznych zdolnych do uwalniania mineralnego fosforu z materii organicznej i nieorganicznej. Wyniki przeprowadzonych badań wskazują, że najważniejszą rolę w odbudowie dostępnej biologicznie puli mineralnego fosforu odgrywają bakteryjne fosfatazy.

Słowa kluczowe: fosfor w wodzie, uwalnianie z materii mineralnego fosforu, bakterie heterotroficzne